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Responses of soil microbial activity to cadmium pollution and elevated CO₂

SUBJECT AREAS:

CLIMATE-CHANGE
ECOLOGY

SUSTAINABILITY

SOIL MICROBIOLOGY

MICROBIAL ECOLOGY

Yi Ping Chen¹, Qiang Liu^{1,2}, Yong Jun Liu³, Feng An Jia¹ & Xin Hua He⁴¹SKLLQG, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an 710075, China, ²University of Chinese Academy of Sciences, Beijing 100049, China, ³College of Environmental Science, Xi'an University of Architecture and Technology, Xi'an 710062, China, ⁴Elizabeth Macarthur Agricultural Institute, Department of Primary Industries, Menangle, NSW 2568, Australia.Received
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6 March 2014Correspondence and
requests for materials
should be addressed to
Y.P.C. (chenyp@
ieecas.cn)

To address the combined effects of cadmium (Cd) and elevated CO₂ on soil microbial communities, DGGE (denaturing gradient gel electrophoresis) profiles, respiration, carbon (C) and nitrogen (N) concentrations, loessial soils were exposed to four levels of Cd, i.e., 0 (Cd₀), 1.5 (Cd_{1.5}), 3.0 (Cd_{3.0}) and 6.0 (Cd_{6.0}) mg Cd kg⁻¹ soil, and two levels of CO₂, i.e., 360 (aCO₂) and 480 (eCO₂) ppm. Compared to Cd₀, Cd_{1.5} increased fungal abundance but decreased bacterial abundance under both CO₂ levels, whilst Cd_{3.0} and Cd_{6.0} decreased both fungal and bacterial abundance. Profiles of DGGE revealed alteration of soil microbial communities under eCO₂. Soil respiration decreased with Cd concentrations and was greater under eCO₂ than under aCO₂. Soil total C and N were greater under higher Cd. These results suggest eCO₂ could stimulate, while Cd pollution could restrain microbial reproduction and C decomposition with the restraint effect alleviated by eCO₂.

Atmospheric carbon dioxide (CO₂) concentration has increased from 280 to 380 ppm since the industrial revolution and is projected to reach 420 to 936 ppm at the end of 21st century depending on scenarios¹. The rapid increase of CO₂ has drawn much attention because it closely relates to global climate change. Most of the attention has focused on the responses of aboveground and belowground processes as elevated CO₂ (eCO₂) impacts numerous biogeochemical processes in both natural and agricultural ecosystems^{1,2}.

Soil organic carbon (SOC) pool is three times greater than C stored in the atmosphere or vegetation biomass². It is therefore imperative to understand how eCO₂ impacts soil C dynamics and the associated underlying mechanisms. Studies have demonstrated that eCO₂ could affect soil microbial communities, C and nitrogen (N) cycling processes^{3–5}. However, how eCO₂ could affect C and N dynamics is of much uncertainty as the responses of soil microbial communities to eCO₂ could be positive, negative or neutral^{6–10}.

Soil microorganisms play vital roles in soil C and N transformations and nutrient cycles and thus affect soil biological, chemical, and physical properties. For instance, the activity of soil microorganisms and microbial community composition determine not only soil C sequestrations and emissions, and the decomposition and accumulation of soil organic matter^{11,12}, but also N₂-fixation, nitrification and denitrification, and the accumulation of plant available NH₄⁺ and NO₃⁻¹³. However, limited information is available to the effect of eCO₂ on soil bacterial and fungal abundance, community structure, and in turn C and N dynamics.

Furthermore, global climate change like elevated CO₂ is happening along with other environmental issues, including heavy metal pollution, which has become a seriously global problem due to anthropogenic activities, such as mining, smelting, and sewage sludge disposal¹⁴. Unlike some organic contaminants, heavy metals are often accumulated in living organisms and not biodegradable in the environment. Many heavy metals, including cadmium (Cd), are toxic to soil microbes, which affect soil biogeochemical processes, such as soil organic matter (SOM) decomposition, through altering microbial biomass and activity^{14,15}. Under climate change, the effects of Cd on soil microbial activity may become more complex as potential interactions between climate factors and excessive Cd in soil. However, the interactive consequence of CO₂ and Cd on soil microbial community has seldom been investigated.

In this study, loessial soils were treated with four levels of Cd, i.e., 0 (Cd₀), 1.5 (Cd_{1.5}), 3.0 (Cd_{3.0}) and 6.0 (Cd_{6.0}) mg Cd kg⁻¹ soil, and two levels of CO₂, i.e., 360 (aCO₂) and 480 (eCO₂) ppm. Soil bacterial and fungal abundance, community structure, CO₂ emission, C and N were measured to determine how soil Cd pollution and elevated CO₂ separately or in combination could impact soil microbial community and C and N accumulation.

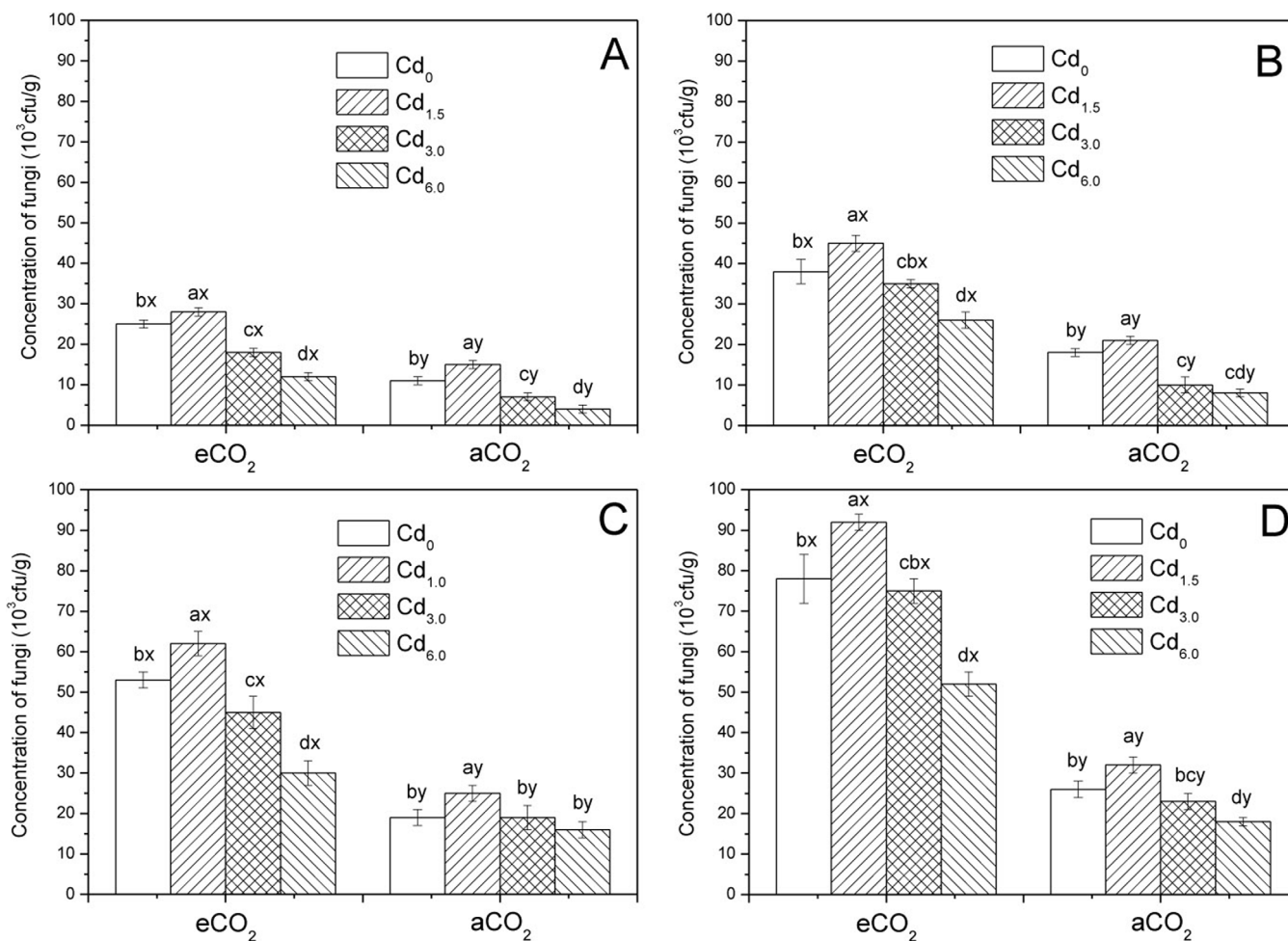


Figure 1 | Effects of cadmium and CO₂ on soil fungal abundance after 2 weeks (A), 4 weeks (B), 6 weeks (C) and 8 weeks (D) under 0 (Cd₀), 1.5 (Cd_{1.5}), 3.0 (Cd_{3.0}) and 6.0 (Cd_{6.0}) mg Cd kg⁻¹ soil and ambient CO₂ (360 ppm, aCO₂) or elevated CO₂ (480 ppm, eCO₂). Data (means ± SE, *n* = 3) followed by different letters between Cd treatments for the same CO₂ treatment (a, b, c, d) or between aCO₂ and eCO₂ for the same Cd treatment (x, y) are significantly different at *P* < 0.05 according to Mann-Whitney multiple comparisons.

Results

Microbial community. The abundance of soil fungi was affected not only by Cd but also by CO₂ concentrations (Fig. 1). Fungal abundance generally linearly increased with incubation time over the eight weeks of Cd exposure under all treatments. Fungal abundance was much greater under eCO₂ than under aCO₂, regardless of Cd levels. Compared to the non-Cd control, fungal abundance was significantly increased under Cd_{1.5}, but decreased under Cd_{3.0} and Cd_{6.0}, regardless of CO₂ levels.

Soil bacterial abundance was gradually decreased with increase of Cd concentrations. Its abundance was lower under aCO₂ than under eCO₂ at the same Cd concentration. Bacterial abundance was decreased after 2 to 4 weeks and was lowest at 4 week over the eight weeks of Cd exposure under both aCO₂ and eCO₂ (Fig. 2). But soil bacterial abundance began to increase after 4 weeks and was highest at 8 weeks of incubation.

The triplicate running of DGGE profiles was highly reproducible so only one DGGE profile was displayed (Fig. 3). After 4 weeks of incubation, 10 bands under eCO₂ while 8 bands under aCO₂ were obviously observed, irrespective of Cd concentrations (left panel in Fig. 3). Among these bands, 4 distinctive bands were generated (the left-towards red arrows in the left panel) and 2 bands were disappeared (the right-towards red arrows in the left panel) under eCO₂ than under aCO₂. Furthermore, after 8 weeks of incubation, 8 bands under eCO₂ while 6 bands under aCO₂ were obviously observed,

irrespective of Cd concentrations (right panel in Fig. 3). Among these bands, 3 distinctive bands were generated (the left-towards red arrows in the right panel) and 1 band was disappeared (the right-towards red arrows in the right panel) under eCO₂ than under aCO₂. In addition, there were visual differences in DGGE band width between two incubation durations (4 and 8 weeks), while there seemed no distinctively visual differences between Cd₀ and other Cd concentrations (no measurements were done to determine the width). Therefore, the DGGE profiles, e. g., the soil microbial community structure, were altered under eCO₂ and cadmium pollution over the 8 weeks of soil incubation.

Soil C and N dynamics. The CO₂ emission showed a decrease trend with the increase of Cd concentrations under either eCO₂ or aCO₂ (Fig. 4). The cumulative soil CO₂ effluxes were generally significantly greater under eCO₂ than under aCO₂ over the 8 weeks incubation, regardless of Cd treatment levels.

Concentrations of soil C and N were greater in soils treated with higher concentrations of Cd at both CO₂ levels. Significantly higher concentrations of soil C and N were observed under aCO₂ than under eCO₂, regardless of Cd treatments (Fig. 5a and 5b).

Discussion

Due to excessive emission of industrial waste, wastewater irrigation and serious abuse of chemical fertilizers and pesticides, numerous

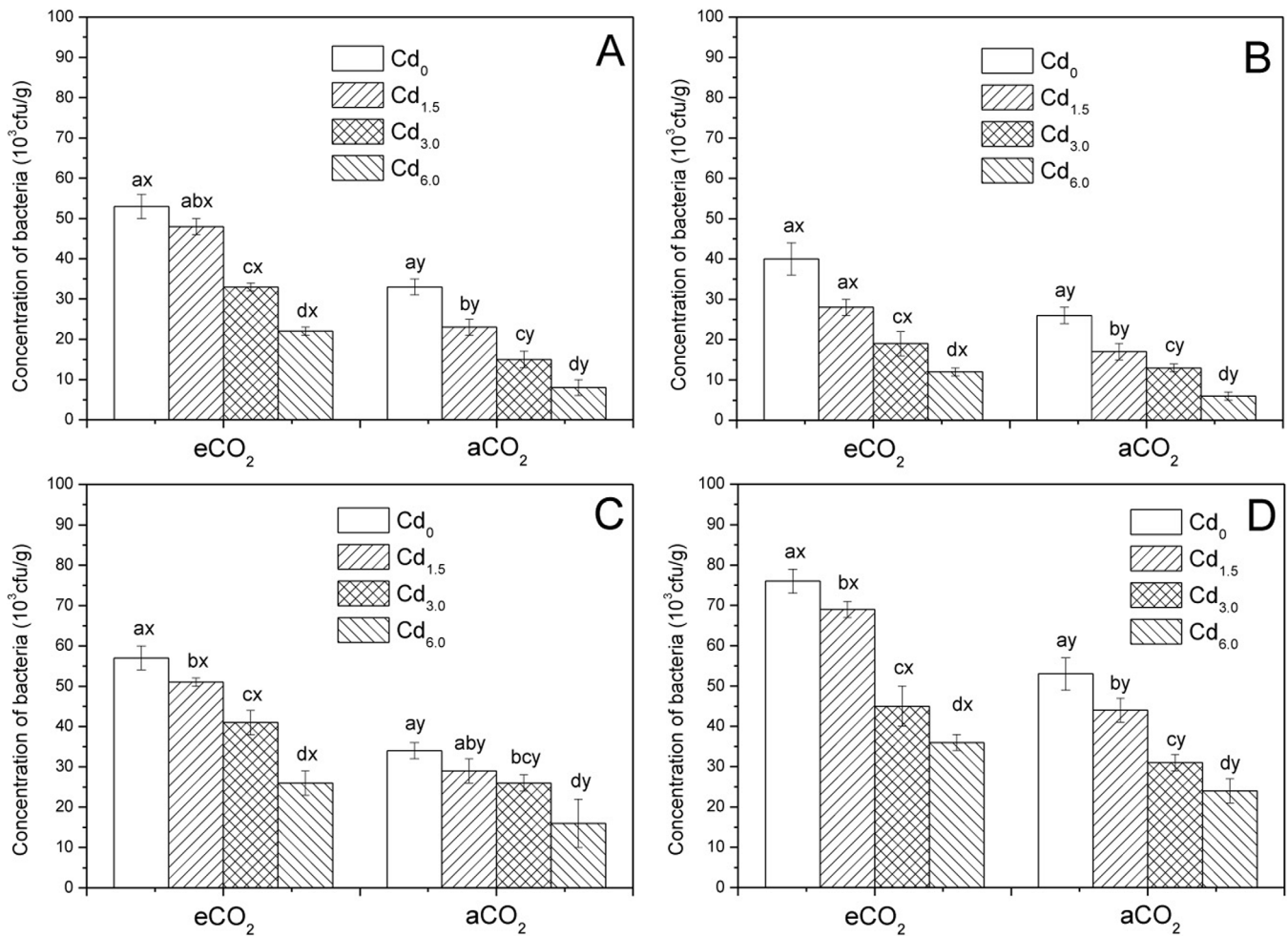


Figure 2 | Effects of cadmium and CO₂ on soil bacterial abundance after 2 weeks (A), 4 weeks (B), 6 weeks (C) and 8 weeks (D) under 0 (Cd₀), 1.5 (Cd_{1.5}), 3.0 (Cd_{3.0}) and 6.0 (Cd_{6.0}) mg Cd kg⁻¹ soil and ambient CO₂ (360 ppm, aCO₂) or elevated CO₂ (480 ppm, eCO₂). Data (means ± SE, *n* = 3) followed by different letters between Cd treatments for the same CO₂ treatment (a, b, c, d) or between aCO₂ and eCO₂ for the same Cd treatment (x, y) are significantly different at *P* < 0.05 according to Mann-Whitney multiple comparisons.

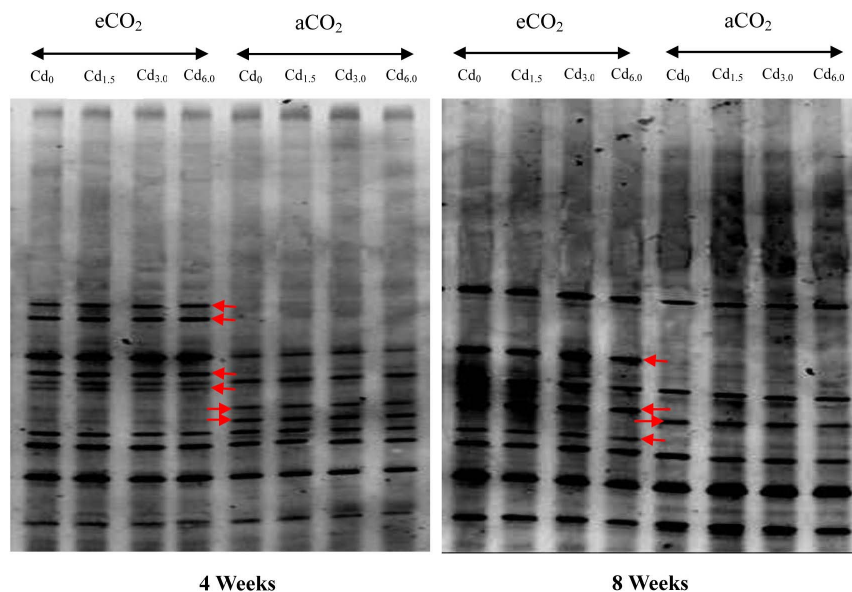


Figure 3 | Effects of cadmium and CO₂ on soil bacterial community structure (DGGE profiles of PCR products from 16S rDNA extractions) after 4 weeks and 8 weeks under 0 (Cd₀), 1.5 (Cd_{1.5}), 3.0 (Cd_{3.0}) and 6.0 (Cd_{6.0}) mg Cd kg⁻¹ soil and ambient CO₂ (360 ppm, aCO₂) or elevated CO₂ (480 ppm, eCO₂). Red arrow showed differences in DGGE bands between aCO₂ and eCO₂ treatments.

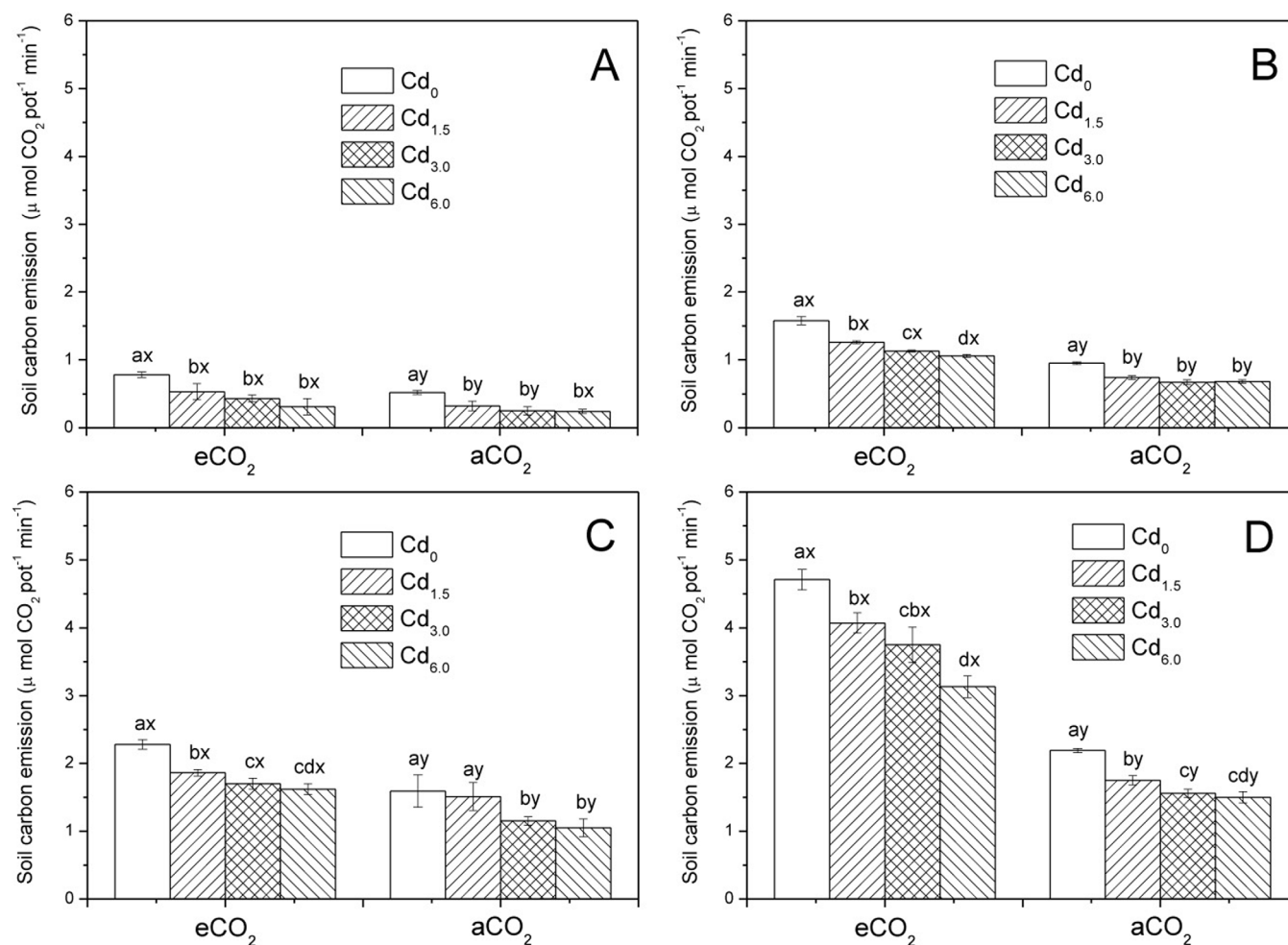


Figure 4 | Effects of cadmium and CO₂ on soil cumulative carbon emission after 2 weeks (A), 4 weeks (B), 6 weeks (C) and 8 weeks (D) under 0 (Cd₀), 1.5 (Cd_{1.5}), 3.0 (Cd_{3.0}) and 6.0 (Cd_{6.0}) mg Cd kg⁻¹ soil and ambient CO₂ (360 ppm, aCO₂) or elevated CO₂ (480 ppm, eCO₂). Data (means ± SE, *n* = 3) followed by different letters between Cd treatments for the same CO₂ treatment (a, b, c, d) or between aCO₂ and eCO₂ for the same Cd treatment (x, y) are significantly different at *P* < 0.05 according to Mann-Whitney multiple comparisons.

heavy metals have been released into the environment¹⁶. One fifth of total cultivated land in China was polluted by heavy metals, such as arsenic (As), cadmium (Cd), chromium (Cr) and lead (Pb)¹⁷. Heavy metal pollution has been a threat to the earth system. Previous studies have showed that either short-term or long-term exposure to heavy metals could result in changing of soil microbial communities, or decrease in diversity and activities^{12–15}. Moreover, fungi are more tolerant to heavy metals than bacteria¹⁸. Our results in loessial soil were consistent with those previous studies¹⁸. Bacteria were more sensitive to Cd stress than fungi because the low (1.5 mg Cd kg⁻¹ soil) treatment restrained the bacteria reproduction but improved fungal reproduction (Fig. 1 and 2). With the increase of Cd concentrations, the fungal and bacterial abundance were gradually decreased under normal CO₂ (Fig. 1 and 2). Moreover, the alteration of DGGE profiles might provide direct evidence that the microbial communities might have been influenced by elevated CO₂, but not by cadmium pollution at the tested Cd concentrations (Fig. 3). Consequently, no matter whether the soil was incubated under the ambient CO₂ or the elevated CO₂ concentration, soil respiration was restrained while concentrations of soil C and N were increased (Fig. 4 and 5), indicating that Cd pollution could alter soil biogeochemical cycling.

Elevated CO₂ have various impacts on biogeochemical processes in terrestrial ecosystems. There were some different viewpoints on the effect of elevated CO₂ on soil microbial abundance. For instance,

in free-air CO₂ enrichment experiment no significant changes were observed in the relative abundance or composition of fungi¹⁹. On the contrary, the fungal biomass in a chaparral ecosystem was significantly increased under 550 ppm elevated CO₂²⁰, and the relative abundance of fungi in a scrub-oak ecosystem was greater under elevated CO₂ than under ambient CO₂³. Elevated CO₂ could stimulate microbial growth, especially bacteria, and lead to substantial changes in the activity and structure of soil microbial communities²¹. Recently, analyses of 16S rRNA genes showed that soil microbial community structures were significantly changed by elevated CO₂²². Our experiments also demonstrated the bacterial and fungal abundance were increased by eCO₂ regardless of Cd levels. Compared with the same Cd concentration, elevated CO₂ significantly altered the bacterial and fungal abundance, the bacterial DNA band, and decreased the concentrations of soil C and N, resulting in changes in soil microbial community and soil biogeochemical element cycling. These results support that elevated CO₂ could result in a loss of soil carbon⁴.

Elevated CO₂ and Cd exhibited opposite effects on soil microbial communities. Cd pollution restrained microorganism's growth and high concentration of Cd could decrease microbial biomass and activities. As a result, less SOM could be utilized and thus less CO₂ was released from soil, which in turn had more C and N retained in the soil. Moreover, with an increase of Cd concentration, the negative effect was more obvious. However, soil microbial relative abundances

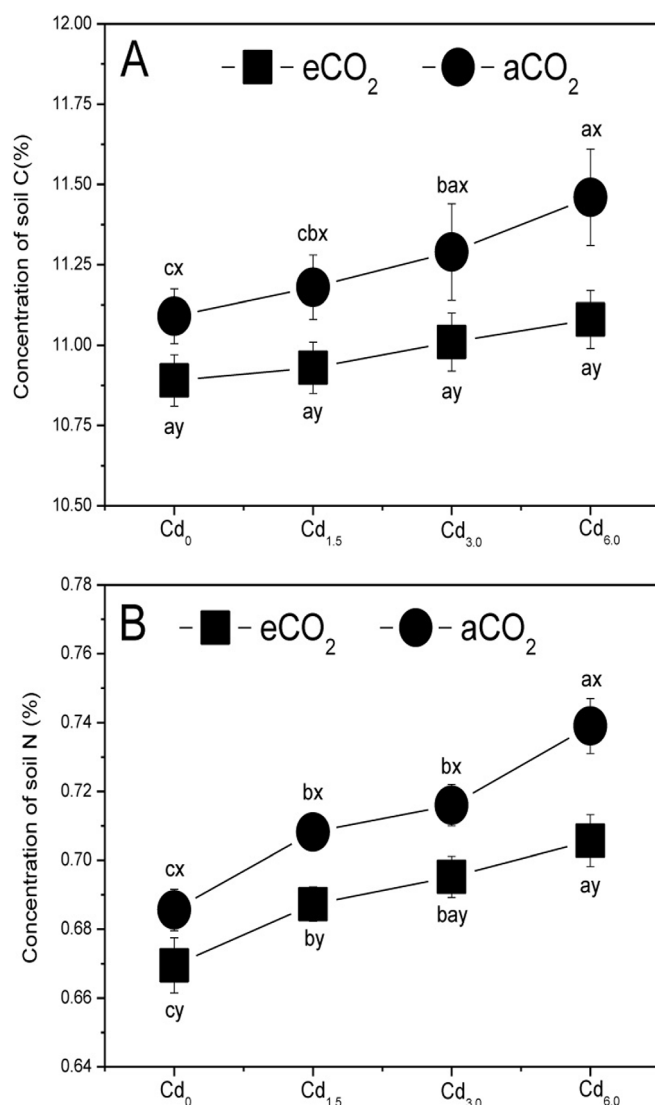


Figure 5 | Effects of 8-weeks cadmium and CO₂ treatments on soil carbon (A) and nitrogen (B) concentrations under 0 (Cd₀), 1.5 (Cd_{1.5}), 3.0 (Cd_{3.0}) and 6.0 (Cd_{6.0}) mg Cd kg⁻¹ soil and ambient CO₂ (360 ppm, aCO₂) or elevated CO₂ (480 ppm, eCO₂). Data (means ± SE, *n* = 3) followed by different letters between Cd treatments for the same CO₂ treatment (a, b, c, d) or between aCO₂ and eCO₂ for the same Cd treatment (x, y) are significantly different at *P* < 0.05 according to Mann-Whitney multiple comparisons.

and activities of soil carbon-degrading enzymes in soils exposed to an elevated CO₂ might have been increased, leading to an enhanced degradation of soil organic matter than soils exposed to ambient CO₂, which in turn consumed more C and N in soil. Therefore, a combined effect of Cd and eCO₂ could display antagonisms on terrestrial C pool by altering soil microbial community.

In conclusion, higher Cd exposure to soil could lead to a decrease of both bacterial and fungal abundance, and hence an increasing soil C and N concentrations. Meanwhile, elevated CO₂ could alter microbial communities and lead to an increase of soil C emission, resulting in a decrease in soil C and N concentrations. These results suggest that eCO₂ could stimulate, while Cd pollution could restrain, microbial reproduction and C decomposition with the restraint effect alleviated by eCO₂. Nevertheless, more research is warranted on the combined effects of Cd pollution and elevated CO₂ on the dynamics of soil C.

Methods

Soil characteristics. Loessial soils at 0–10 cm depth were collected from a field after maize harvest in a suburban of Xi'an, Shaanxi, China (34° 44' N, 109° 49' E). According to Pang et al.²³, the soil pH was 8.3, moisture was 7.8%, soil SOC was 8.9 g C kg⁻¹ and CaCO₃ was 30.11 g kg⁻¹.

Experiment design. The experiment consisted of four Cd and two CO₂ treatments in a completely randomized arrangement. The four Cd treatments were 0 (Cd₀), 1.5 (Cd_{1.5}), 3.0 (Cd_{3.0}) or 6.0 mg Cd kg⁻¹ soil (Cd_{6.0}) and the two CO₂ treatments were ambient (360 ppm, aCO₂) or elevated (480 ppm, eCO₂). Each treatment had three replicates, for a total of 24 replicates. The soil was put in 24 growth pots. Each pot (15 cm diameter and 10 cm height) contained 1.0 kg soil. The CO₂ concentrations were monitored and controlled by a meter (output flux: 25 L min⁻², 15 MPa pressure; Shanghai Yichuan Meter Co., Shanghai, China). Cd was applied as CdCl₂·2.5H₂O (99%, Tianjin Fuchen Chemical Co., Tianjin, China), which was firstly dissolved in distilled water and then thoroughly mixed with soil. The Cd-containing soils were continuously incubated under 25 ± 1 °C and 70% of relative humidity over 8 weeks in a plant growth chamber.

Soil microbial community structure. The number of bacteria and fungi was counted using the plate counting technique²⁴. Briefly, approximately 10 g soils from each pot were put into a 300 ml Erlenmeyer flask containing 90 ml sterilized potassium phosphate buffer (0.35 g KH₂PO₄, 0.65 g K₂HPO₄ and 0.10 g MgSO₄ in 1.0 liter water), centrifuged at 250 rpm for 15 min and the soil extraction solution was then diluted into 100-fold. Control plates minus soil suspensions were used to check potential contamination. The total number of bacteria was cultured with an agar medium (including 5 g beef, 10 g peptone, 5 g NaCl, 20 g agar and 0.1 g cycloheximide per 1,000 ml distilled water) for 48 h at 37 °C. The total number of fungi was cultured with an agar medium (including 10 g glucose, 5 g peptone, 1 g KH₂PO₄, 0.5 g MgSO₄·7H₂O, 20 g agar and 0.1 g chloramphenicol per 1,000 ml distilled water) for 72 h at 25 °C. The colony concentrations were expressed as colony-forming units per gram soil (CFU/g)²⁴.

DNA extraction and PCR-DGGE analysis. Analyses of bacterial community structure were performed with incubated soils at week 4 and week 8, respectively. Using a Fast DNA Spin Kit (Bio 101, USA), the extraction of soil bacterial DNA was tripled (1.0 g soil each) from each treatment according to the manufacturer's bead-beating procedure. The yield and quality of extracted DNA was validated by the 1.0% (w/v) agarose gel electrophoresis with ethidium bromide staining. The bacterial 16S rDNA genes were amplified with the primer EUB341F (5'-CCT ACG GGA GGC AGC AG-3') with a GC clamp (CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC G) at the 5' end and the primer EU500R (5'-GTA TTA CCG CGG CTG CTG G-3')²⁵. Using 50 µl reaction volumes the PCR amplification was carried out with an iCycler Thermal Cycler (Bio-Rad, USA). A final 50 µl reaction mixture contained 1–10 ng DNA extracts as template, 5 µl of 10 × PCR buffer (minus Mg²⁺), 15 pmol of each primer, 1.25 µl unit Taq polymerase, 200 µmol deoxyribonucleoside triphosphate, and 3 µl MgCl₂ (25 mmol). A touchdown temperature profile was applied as the annealing temperature decreased from 65 °C to 55 °C after 20 cycles with a 0.5 °C decline from each cycle, and then the mixture was kept at 55 °C for further 10 cycles. An aliquot of 5 µl PCR products was then run by 1.0% agarose gel electrophoresis with ethidium bromide staining prior to the denaturing gradient gel electrophoresis (DGGE), which was conducted with a Bio-Rad Dcode™ Universal Mutation Detection System (Bio-Rad, USA). The generated PCR product was loaded on 0.8 mm thick polyacrylamide gel (10% acrylamide/bisacrylamide, w/v = 37.5/1) under a 30% to 50% of urea and formamide (100% corresponds to 7 mol urea and 40% formamide, w/v) denaturing gradient that was increased in the electrophoresis running direction. The electrophoresis was run for 5 h under 60 °C and 150 V. After the run, the gels were stained with 1 × TAE (containing 0.5 mg ml⁻¹ ethidium bromide) for 20 min, rinsed with distilled water thoroughly, and then photographed.

Measurements of soil total carbon and nitrogen. Soils from all 8-week incubation pots were air-dried to a constant weight and sieved through 1.0 mm mesh. Total carbon and nitrogen were determined according to the dichromate oxidation²⁶ and the H₂SO₄ titrate method²⁶, respectively.

Soil CO₂ efflux measurements. After incubation for 2, 4, 6 and 8 weeks, the pot was placed into a PVC (polyvinyl chloride) collar (14.5 cm height and 20.3 cm inside diameter) and the bottom was completely sealed. Soil CO₂ efflux rates (µ mol CO₂ pot⁻¹ min⁻¹) between 12:00 pm and 15:00 pm were quantified by a LI-8100 soil CO₂ flux system (LI-COR Inc., Lincoln, NE, USA), which was equipped with a portable chamber (Model 8100-103) being placed into the PVC collar. The CO₂ efflux rate was then automatically calculated from exponential regression of increasing CO₂ concentrations over 3-min duration. Each measurement for a pot was repeated 3 times and 3 repetition experiments were performed in one day.

Data analysis. Data (means ± SE) were subjected to one-way analysis of variance (ANOVA) and significant differences between treatments were compared using the Mann-Whitney multiple comparison at *P* < 0.05. Statistical analyses were performed using the SPSS16.0 (SPSS Inc., Chicago, IL, USA).



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Author contributions

Y.C. designed experiments, conducted CO₂ emission measurements, data analyses and wrote the manuscript. Q.L. performed experiments in soil carbon and nitrogen. Y.L. performed experiments in DGGE profiles. F.J. did experiments in bacterial and fungal abundance. X.H. analyzed data and rewrote the manuscript. All authors reviewed the manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

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